CONTRACT NO.: DAMD17-87-C-7235

TITLE: (U) DEVELOPMENT OF ADVANCED METHODS BASED ON STABLE ISOTOPE TECHNOLOGY FOR STUDIES OF EXERCISE IN HEAT

PRINCIPAL INVESTIGATOR: MORTEZA JANGHORBANI

PI ADDRESS: University of Chicago
Department of Medicine/GI/CNRU
5841 S. Maryland Avenue
Box 223
Chicago, Illinois 60637

REPORT DATE: April 15, 1989

TYPE OF REPORT: Midterm

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK
FREDERICK, MARYLAND 21701-5012

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: University of Chicago; 6b. OFFICE SYMBOL (If applicable): N/A	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): 970 E. 58th Street, Chicago, IL 60637	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-87-C-7235
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3M1-61102AS15; TASK NO. CA; WORK UNIT ACCESSION NO. 061

11. TITLE (Include Security Classification)
Development of Advanced Methods Based on Stable Isotope Technology for Studies of Exercise in Heat

12. PERSONAL AUTHOR(S)
Morteza Janghorbani, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Midterm	FROM 9/28/87 TO 3/31/89	April 15, 1989	19

16. SUPPLEMENTARY NOTATION
Cont'd From Pg. 5

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	05		Stable Isotopes, Exercise, Heat
06	04		RA 3

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Stable isotope methods were developed for precise and accurate measurement of isotopes of bromine, magnesium, and potassium in tissues. The methods were applied to the measurement of extracellular fluid volume using the swine model, and to exchangeable pool sizes of magnesium and potassium using the rat model. It was shown that the bromine method could be combined with the stable isotope methodology for the measurement of total body water to investigate body water compartments. It was shown that the magnesium method could be used to investigate changes in exchangeable body magnesium in the rat made magnesium deficient by dietary manipulation. The implications of the use of stable isotopes of magnesium and potassium are being explored further. Keywords: Heat Stress Physiology

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mary Frances Bostian	301-663-7325	SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_____ Where copyrighted material is quoted, permission has been obtained to use such material.

_____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

_____ Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

_____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

_____ For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

_____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Mirtha Jaylin 4/15/89

PI Signature Date

All

TABLE OF CONTENTS

FOREWORD 3

INTRODUCTION 5

METHODS 5

1. Development of Analytical Methods 5
2. Applications 10

CONCLUSIONS 13

REFERENCES 18

TABLES

1. Summary of Data Establishing Accuracy of Isotopic Analysis for Stable Isotopes of Mg in Matrices of Interest 8

2. Measurement Precision and Accuracy for $MR_{41/39,c}$ in Biological Materials, Scheme III 11

3. Ion Beam Intensity Ratio ($MR_{41,39,c}$) for Biological Matrices, Scheme IV 11

4. Comparison of Exchangeable Mg (Mg_e), Whole Carcass Mg and its Components in Animals Fed Three Levels of Mg . . 17

FIGURES

1. Time course of the change in $MR_{25/24}$ 13

2. The time course of ^{25}Mg exchange after a single I.P. injection of ^{25}Mg (3.62 $\pm$ 0.08 mg) for the soft tissues sampled 15

INTRODUCTION

Exercising in heat may perturb the normal state of electrolyte balance due to excessive losses via perspiration. This may lead to the development of an electrolyte deficit and or peturbation of normal compartmentation within the intracellular/extracellular spaces. Little quantitative information is available on these issues due to lack of accurate methods for quantitative investigations.

The overall objectives of this contract are to develop safe and non-invasive methods, based on the stable isotope tracer approach, to permit investigations of dynamics of electrolyte compartmentation in humans and under the conditions of physiologic relevance to exercise in heat. While broad in scope, the present emphasis of the work is on the following: studies of total body water and its compartments (extracellular/intracellular spaces), direct measurement of body potassium, and studies of body magnesium.

Aside from the stable isotope methodology for the measurement of total body water, the methods required for this work were not available at the time of initiation of this contract. These methods are based on emerging analytical technology. Their development has become possible only due to recent availability of new mass spectrometric instrumentation (1).

For this reason, two major aspects of the work are considered in the context of this contract: development of analytical methods for accurate measurement of stable isotopes of selected electrolytes, and demonstration of the feasibility of their use using animal models. Initially, we focussed on the following specific problems: simultaneous measurement of total body water and extracellular fluid volume, measurement of body potassium, and measurement of body magnesium. Each of these three problems is an important issue about which little quantitative information is available in the context of exercising in heat.

METHODS

The work carried out to date under this contract will be described under two general headings: development of analytical methods, and studies of feasibility with animal models.

1- Development of Analytical Methods

Stable isotope methods were developed successfully under this contract for the three electrolytes of interest. Methods for two of these electrolytes (Br, Mg) have been published (2, 3)

the manuscript for the third electrolyte (K) has been submitted for publication (6). This is the first time that such methodology has become available.

a- Analytical Chemistry of Stable Isotopes of Bromine

Bromine consists of two stable isotopes in nature: ^{79}Br and ^{81}Br. The method of inductively coupled plasma mass spectrometry (2) is the first reported method for application to the dynamics of Br/Cl transport in man. In addition, it also provides the most accurate method available for the measurement of Br-space utilizing the concept of Br-dilution together with in vitro isotope dilution analysis.

The method as now routinely used is capable of accurate quantitative analysis of both stable isotopes of Br present at natural levels or higher in human plasma with overall accuracy of ~1%.

The following account briefly describes this method for specific application to the issue of accurate measurement of Br-space. The method involves administration of a pharmacologic dose of natural bromine, followed by measurement of its dilution in the extracellular space.

Scheme I-*SCHEME for Analysis of Br in plasma*

1- Place 5 ml sample in dialysis tubing (Spectro/Por #132680, MW Cutoff 12000-14000, Spectrum Medical Industries, Los Angeles, CA) tie both ends in knots to prevent leaking. Add appropriate amount of highly enriched ^{81}Br as in vitro spike.
2- Place dialysis tubing inside polyethylene bag (Ziplock) containing 50 ml DI-water; close using dialysis closures.
3- Place bags on horizontal laboratory shaker; shake for 1.5-2.0 hours.
3- Decant outer solution into beaker; boil until volume is a few milliliters.
4- Apply to cation exchange column (Dowex 50-x8, 100-200 mesh, 15x75 mm, Bio.Rad Laboratories, Richmond, CA).
5- Elute with 25 ml DI-water ; reduce volume if necessary by boiling.
6- Run on ICP/MS for $^{81}Br/^{79}Br$.

From the measurement of the ratio $^{81}Br/^{79}Br$ ($=MIR_{81/79}$) calculate the Br content of the plasma sample using the following expression:

$$X=[^{81}Br^{*}-(MIR_{81/79})x(^{79}Br^{*})]/[0.5007x(MIR_{81/79}-0.9974)]$$

X: μg Br in sample
$^{81}Br^*$, $^{79}Br^*$: μg isotope added to sample as *in vitro* spike
0.5007, 0.9974: constants related to natural isotopic composition of Br.

b- Analytical Chemistry of Stable Isotopes of Magnesium

There are three stable isotopes of Mg: ^{24}Mg, ^{25}Mg, and ^{26}Mg. The method described here is the first application of inductively coupled plasma spectrometry to accurate measurement of stable isotopes of Mg in biological materials (3). It is capable of quantitative measurement of the three stable isotopes of Mg with accuracy of ~1%. The isotope ratios $^{25}Mg/^{24}Mg$ ($=MIR_{25/24}$) and $^{26}Mg/^{24}Mg$ ($=MIR_{26/24}$) can be measured with precision and accuracy of ≤1.0%.

Scheme II- *SCHEME OF ANALYSIS FOR Mg*

1- Digest sample (0.5-1.5 g) with Conc. HNO_3(20 mL)+H_2O_2(20 mL) +Conc. H_2SO_4(1 mL) [for urine 2 mL, do not digest, go to step 4] {Spike with highly enriched ^{25}Mg (3-5 times its expected content) if quantitative isotopic anlaysis is desired}
2- Evaporate to small drop; add water and repeat evaporation step 2-3 times (to remove as much residual acid as possible)
3- Dilute with DI-H_2O to approximate Mg concentration of 5-20 μg/mL
4- To 1 mL of solution add 25 μL of 1000 μg/mL $CaCl_2$ (as carrier)
5- Add 1 mL of 2% $NH_4H_2PO_4$ and 2.5 mL of 30% NH_4OH. Add more base if pH<8.
6- Let sit on ice for ~1 hr., refrigerate overnight
7- Centrifuge for 15 min. at 5000g, 4°C
8- Discard supernatant; dissolve pellet with 200 μL conc. HNO_3
9- Dilute sufficiently to [Mg]~0.05 μg/mL; run on ICP-MS with appropriate isotope calibration standards

The accuracy of isotopic analyses in various matrices of interest was tested by the application of *in vitro* isotope dilution (^{26}Mg as *in vitro* isotope diluent, ^{24}Mg as reference isotope) to subsamples of rat tissues (Table 1), or standard reference materials (Bovine Liver: NBS SRM 1577a; Bone: IAEA Animal Bone H-5), and comparison of the

results with both measurements carried out on the subsample digests using atomic absorption spectrophotometry and the certified values, where applicable. The expression employed for calculation of Mg content of subsamples with the *in vitro* isotope dilution procedure is:

$$W_{Mg}=[W_{Mg-26}^{*}-(MIR_{26/24}).W_{Mg-24}^{*}]/[0.7795(MIR_{26/24})-0.1177]$$

where:

W_{Mg}: weight of Mg in the subsample, μg

W_{Mg-26}^{*}, W_{Mg-24}^{*}: weights of respective isotopes from the spike, μg

$MIR_{26/24}$: Mass Isotope Ratio for the subsample

0.7795, 0.1177: constants relating natural abundances of ^{24}Mg and ^{26}Mg to Mg

The results of these tests have been summarized in Table 1.

Table 1- Summary of Data Establishing Accuracy of Isotopic Analysis for Stable Isotopes of Mg in Matrices of Interest

Matrix	Mg Concentration (μg/g)		
	in vitro isotope dilution[1]	AAS[1]	Certified Value
SRM 1577a	617. ± 4.	592. ±17.	600.±15.
IAEA H-5	3585. ±16.	3752. ±29.	3550.±90.
Rat Liver	237. ± 2.	237. ± 4.	
Rat Muscle	300. ± 4.	317. ± 5.	
	306. ± 4.		
Rat Plasma	26.6± 1.2	25.2± 1.8	
Rat RBC	45.3± 0.7	45.4± 0.6	
	46.0± 0.8		

[1]mean±1SD (n=4). AAS: atomic absorption spectrometry

Based on these data, isotope ratios for stable isotopes of Mg in biological matrices can be measured routinely with analytical precision (%RSD) of 1% or better. This can be done with a relatively simple requirement for chemical separation and a high sample throughput. Because of the existence of three stable isotopes of Mg, the method of *in vitro* isotope dilution can be readily applied to

samples resulting from *in vivo* labeling studies with a single stable isotope, permitting high accuracy quantitative isotopic analysis. This new method is likely to prove suitable for a number of stable isotope tracer investigations of metabolism of Mg, studies for which no alternative is available.

The important question of achieving isotope ratio measurement precision consistently at or near 0.1% remains yet to be addressed. This issue should be considered a high-priority problem in the context of Mg research. However, its resolution will require considerable insight into both fundamental aspects of ICP-MS processes as well as issues related to matrix composition of these complex materials.

c- Analytical Chemistry of Stable Isotopes of Potassium

This is the first attempt at developing a suitable stable isotope approach to the problem of measurement of exchangeable body potassium (K_e). Because of the high content of potassium in the body, the primary criterion dictating the feasibility of this approach is the precision of isotope ratio measurement for $^{41}K/^{39}K$. Thus, our effort to date has been focussed on the development of measurement procedures which would permit the highest measurement precision that can be achieved with ICP-MS.

Two chemical separation procedures were tested (Scheme III and IV). Scheme III was based on ion exchange chromatography, while Scheme IV utilizes selective precipitation of K (6).

Scheme III- *SCHEME OF ISOTOPIC ANALYSIS FOR K (based on ion exchange chromatography)*

1- Wet ash sample (~100-500 µg K) with conc. HNO_3 (10-15 mL)+H_2O_2 (30%, <5 mL). If desired add enriched ^{41}K.
2- Adjust pH to 7.
3- Apply to cation exchange column (Dowex 50w-x16, 200-400 mesh, capacity 2.6 meq/mL of resin bed; column 1 cm i.d., 5 cm long)
4- Wash column with 40 mL, 0.9 M HCl; discard
5- Elute with 40 mL, 0.9 M HCl; save
6- Measure [K] approximately; adjust with deionized water to [K]~5 µg/mL
7- Analyze for ratio $^{41}K/^{39}K$

Scheme IV- *SCHEME OF ISOTOPIC ANALYSIS FOR K (based on selective precipitation)*

1- Wet ash sample (~100-500 μg K) with conc. HNO_3 (10-15 mL)+H_2O_2 (30%, <5 mL). If desired add enriched ^{41}K.
2- Adjust pH to 7.
3- Add solution of tetraphenylboron (3 mL of 3.5% W/V)
4- Centrifuge (3000 rpm) for 15 min.
5- Suspend precipitate in deionized water (~50 mL); repeat step 4.
6- Add conc. HCl (~2 mL) to precipitate; place in hot water bath until solution becomes clear.
7- Measure [K] approximately; adjust with deionized water to [K]~5 μg/mL
8- Analyze for ratio $^{41}K/^{39}K$

The results of isotope ratio measurements performed on different biological matrices of interest have been summarized in Table 2 for Scheme III and Table 3 for Scheme IV. The data demonstrate two important issues. First, using Scheme III, isotope ratio measurements with overall precision of close to 0.1% were achieved. And secondly, Scheme III was inherently more precise than Scheme IV.

The results from this work clearly demonstrate that the ratio for $^{41}K/^{39}K$ can now be measured with overall precision close to 0.1%. Based on this experimental observation, one can now determine the minimum dose level that is needed to permit determination of K_e with the necessary degree of overall accuracy. Based on theoretical considerations, an *in vivo* isotope enrichment 10X the achieved measurement precision permits determination of K_e with overall accuracy of about 10%. Thus, if the value of 1.76 g K/kg body weight is accepted for body K, a dose of 1-2 mg ^{41}K/kg body weight would be required (present cost of ^{41}K: $20/mg). Based on these considerations, the present method is suitable for application to small animals and to human infants. A further factor of ten improvement in the measurement precision would then permit application of the method to measurement of K_e in human adults.

2- Applications

Three sets of experiments have been/are being carried out with animal models to show the feasibility of stable isotope tracer approaches to a number of important problems of electrolyte physiology. These experiments have focussed on: (a) simultaneous measurement of total body water and bromine space in the miniature swine, (b) measurement of exchangeable pool of magnesium (Mg_e) in the rat, and (c) measurement of exchangeable body potassium (K_e) in the rat. At this writing, these experiments are ongoing and at different stages of completion.

Table 2- Measurement Precision and Accuracy for $MR_{41/39,c}$ in Biological Materials, Scheme III

Matrix	$MR_{41/39,c}$	Precision[1] Instrumental	Intramatrix	Intermatrix
Standard, 5 μg/mL				
	0.07129	.6		
	0 07115	.3		
	0 07112	.6		
	0 07134	.4		
	0.07114	.1	.14	
Urine-1	0.07129	.3		
-2	0 07152	.1		
-3	0.07158	.3	.21	
Liver-1	0.07110	.3		
-2	0.07122	.3		
-3	0.07114	.6	.09	
RBC-1	0.07144	.7		
-2	0 07154	.3		
-3	0.07129	.1	.18	
Plasma-1	0 07117	.8		
-2	0.07111	.3		
-3	0.07118	.3	.05	.25

[1]Instrumental: Coef. Variation for ten sequential measurements
Intramatrix: Coef. Variation for three independent preparations
Intermatrix: Coef. Variation for all biological matrices

Table 3- Ion Beam Intensity Ratio ($MR_{41/39,c}$) for Biological Matrices, Scheme IV

Matrix	$MR_{41/39,c}$	Precision Instrumental	Intermatrix
Standard, 5 μg/mL	0.07088	0.3	
Carcass	0.06959	1.0	
Kidneys	0.07036	1.7	
Heart	0.07014	0.7	
Brain	0.06999	1.6	
Urine	0.07061	2.8	
Muscle	0.06817	2.2	1.3

a- Simultaneous Measurement of Total Body Water/Bromine Space

Our past efforts at development of analytical methodology for the purpose of simultaneous measurement of total body water (TBW) and its compartments have successfully led to the design of an animal protocol by the USARIEM personnel, under the direction of Dr. M. Durkot, using the miniature swine model to demonstrate successful application of the methods to this issue.

The experiment involves simultaneous measurement of TBW and extracellular fluid volume (ECF) using the combination of $H_2{}^{18}O/D_2O/Br$ and multiple *in vivo* labels. The two labels for water are used to show the extent of agreement under the conditions of dehydration, and Br is the marker for ECF.

Three animals have already undergone the experimental protocol successfully. The basic protocol involved initial measurement of TBW/ECF/plasma volume, followed by experimental dehydration, (about 5% of body weight) and repeat measurement of TBW/ECF/Plasma Volume. The results of the experiment are not yet available.

b- Measurement of Mg_e in the Rat Model

Three experiments have now been successfully completed. The focus of these experiments has been to demonstrate the value of the stable isotope tracer approach to the measurement of Mg_e (Exchangeable Mg) for fututre applications to exercise in heat.

1) EXP #1. The purpose of this experiment was to demonstrate that the new method of ICP-MS can be used effectively to investigate the dynamics of isotope transport in different organs and to obtain initial data on tranorgan isotope exchange kinetics (4).

Seven adult male Fischer 344 rats (353±13 g) were used in this experiment. One animal was sacrificed for measurement of baseline values. Each of the remaining six animals received a single dose of highly enriched ^{25}Mg (3.84 mg ^{25}Mg, 3.89 mg Mg, I.P.). Urine was collected every 4 hours for the first 12 hours and then every 12 h until sacrifice. Two rats were sacrificed at 12, 24, and 48 hours after dosing. Plasma, red cells, kidneys, liver, heart, sample of skeletal muscle (thigh), brain and bone were removed for isotopic analysis. Each sample was analyzed for the ratio $^{25}Mg/^{24}Mg$ (3, 4).

Fig 1

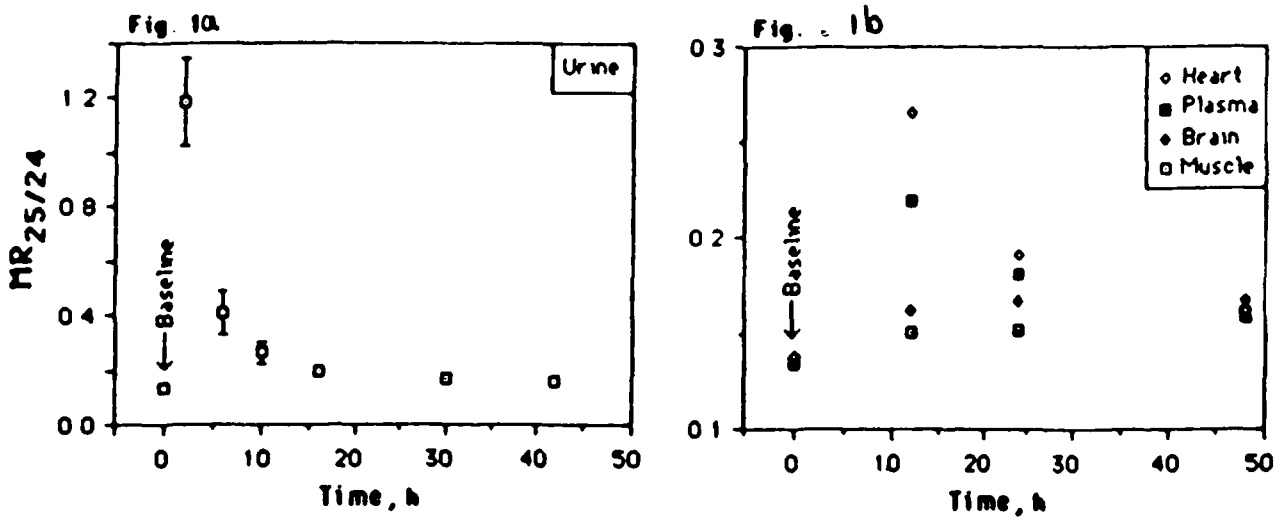


Fig. 1a- Time course of the change in $MR_{25/24}$ of urine after a single dose of ^{25}Mg (3.84 mg). Time (x-axis) was plotted as the midpoint of the collection period. $MR_{25/24}$ designates the measured ion beam intensity ratio for the isotope pair ^{25}Mg and ^{24}Mg.

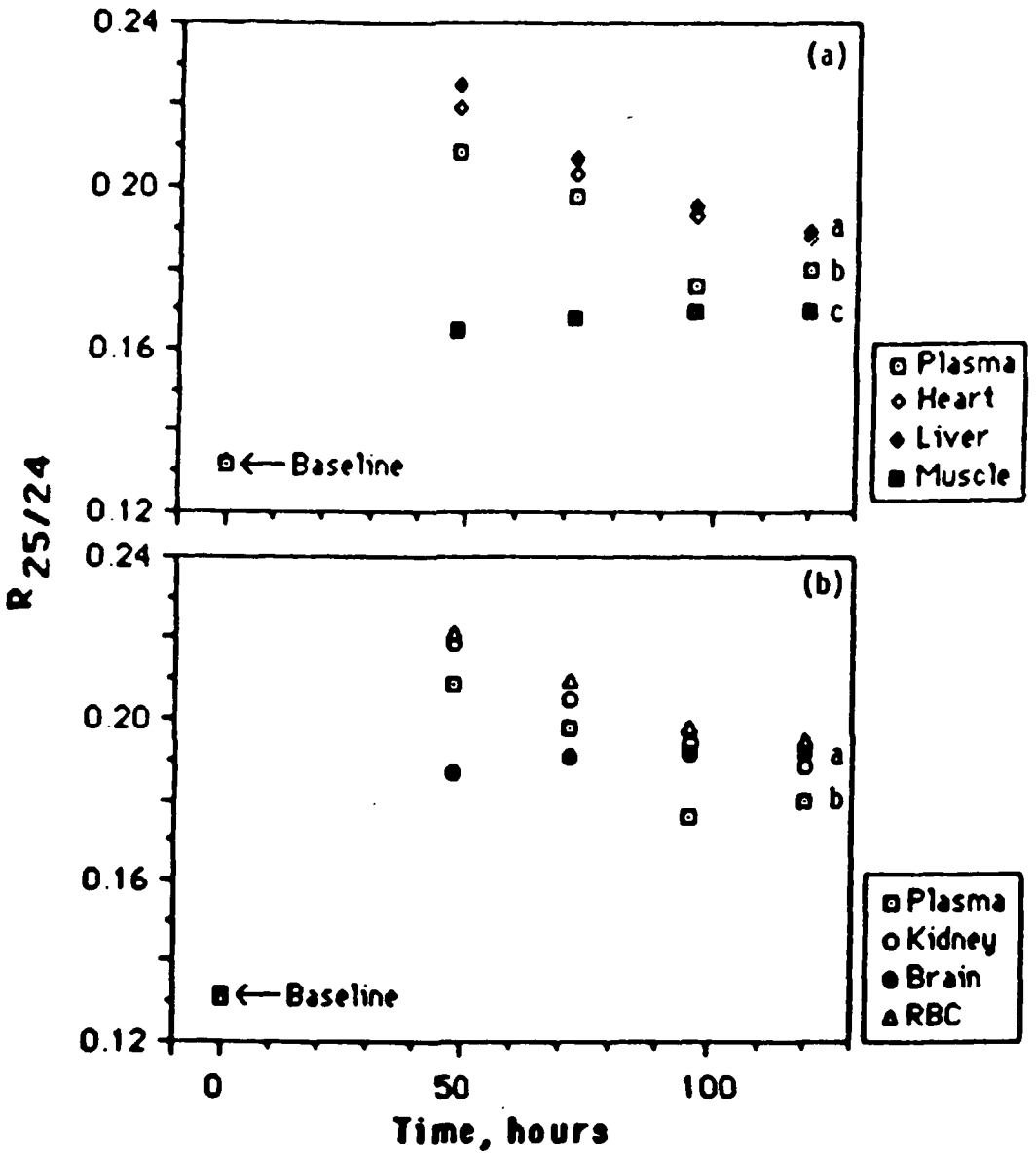
Fig. 1b- Time course of the change in $MR_{25/24}$ for selected tissues. Data represent the $\bar{x}$ of values from two animals.

The primary purpose of this experiment was to provide initial data on the time course of isotope distribution (relative to endogenous Mg) in different organs. The results are summarized in Fig. 1. As shown, urine isotope ratio peaked during the first 4 hours, decreasing thereafter rapidly. However, even at 48 hours postdosing the ratio was 22% higher than the natural ratio. Therefore, from the point of view of accuracy, urine could be employed effectively as sampling compartment, in place of plasma. This would present some important practical advantages for future studies, such as the ability to perform longitudinal studies of Mg_e in the same animal. Isotope ratios for selected tissues are given in Fig. 1b. Compared to plasma, $MIR_{25/24,48h}$=0.161, 48-h soft tissue ratios were: liver 0.160, kidneys 0.156, heart 0.162, brain 0.167, skeletal muscle 0.159, and red cells 0.168. The ratio in urine was the same as for plasma. Therefore, despite the potentially important differences in the details of the time course for various organs, the 48-h value appeared to be the same for all organs (except for bone). This observation indicated then that the concept of *in vivo* stable isotope dilution could be used effectively to determine Mg_e, an important physiological parameter of Mg physiology in relation to exercise in heat.

2) EXP #2. Following the successful outcome of EXP #1, we designed an experiment to evaluate the course of isotope equilibrium in the organs of the rat under the conditions of perturbed Mg intake. The purpose here was to evaluate whether the exchangeability of organ Mg is influenced by the dietary availability of the mineral. This was based on the known homeostatic control of body Mg under the conditions of restricted supply. Two separate experiments were carried out.

a) EXP #2a. The purpose of this experiment was to determine if acute dietary restriction of Mg would alter the time course of isotope equilibrium in the animal (5). Twenty adult rats were fed a Mg-deficient diet supplemented to the level of 0.23% Mg with MgO. The animals were then abruptly switched to the unsupplemented diet (0.0085% Mg). After 3 days on this diet, 4 rats were sacrificed for the measurement of baseline isotope ratios, and the remaining 16 animals were each injected with 3.62 ± 0.08 mg ^{25}Mg (3.66 mg Mg) by I.P. Four animals were sacrificed at each time interval: 48, 72, 96, and 120 hours. Prior to sacrifice, each rat was housed in individual metabolic cage for 12 hours and clean urine was collected. At the time of sacrifice, tissue samples (blood, heart, liver, right kidney, thigh muscle, and brain) were taken for isotopic analysis.

Fig 2



The time course of ^{25}Mg exchange after a single I.P. injection of ^{25}Mg (3.62 ± 0.08 mg) for the soft tissues sampled. The tissue $R_{25/24}$ values for various soft tissues at 48,72, 96 and 120h after isotope dose are shown relative to baseline enrichment. For clarity, only the mean value for 4 rats is shown. Those tissues with $R_{25/24}$ values significantly greater (superscript a) or less than (superscript c) plasma at 120h are indicated, $P < 0.05$.

The results of isotope ratio for $^{25}Mg/^{24}Mg$ have been summarized for this experiment in Fig. 2. In contrast to the isotope ratio data obtained for EXP #1, where essentially complete isotope equilibrium was achieved in all tissues (except for bone) by 48 hours after dosing, the data for EXP #2a clearly demonstrated that under the conditions of acute Mg-restriction the isotopic pattern of organs varied significantly (Fig. 2). Even after 120 hours, the organs did not achieve complete isotopic equilibrium. At this time, isotope ratio for heart, liver, and kidney were similar but about 5% higher than that observed for plasma. Those for brain and red cells were higher than for plasma by ~8%. In contrast, the ratio for skeletal muscle was about 6% lower than for plasma and 11-15% lower than for heart, liver, and kidney. This indicated an important effect of Mg-restriction with respect to Mg distribution within the organs: despite the reported lack of changes in tissue Mg consequent to acute Mg restriction, there appears to be a significant alteration in distribution of newly absorbed Mg (labeled ^{25}Mg) compared with animals maintained on adequate Mg. This may prove to be an important observation in relation to detection of potential consequences of altered Mg status as might occur in exercise in hot climates due to excessve losses from sweat.

b) EXP #2b. Following the observations of EXPs # 1 and 2a, it became clear that alterations in Mg status, whether brought about by dietary restriction or excessive losses due to sweating, may result in important shifts in Mg compartments within the body. Therefore, we carried out a careful experiment with rats fed three levels of dietary Mg for 62 days and then given a single dose of ^{25}Mg in order to establish in more detail the differences in organ Mg contents in comparison with redistribution of the label, and the sensitivity of the proposed pool size method to changes in the exchangeability of body Mg brought about due to dietary Mg restriction.

Eighty one adult rats were utilized in this experiment. Five rats were sacrificed and samples taken for measurement of baseline values. The remaining 76 animals were assigned to three diet groups: a high (H) Mg diet (0.245±0.004% Mg), a control (C) Mg diet (0.051±0.001 Mg) or a Mg deficient (D) diet (0.011±0.001%). Animals from each group were sacrificed at preselected time intervals for Mg analysis of various tissues during the initial 62 days of the experiment. At the end of the 62-days of dietary treatment with three differentt levels of Mg, four animals from each group were placed each in individual metabolic cages for three days of adjustment. After the three days, each animal was injected (I.P.) with 4.66 mg ^{25}Mg (4.71 mg Mg). The animals were returned to their cages and given their respective diets for the remainder of the

experiment. All animals were sacrificed 10 days after administration of the isotope. Prior to sacrifice, 12-hour clean urine was obtained for determination of isotope ratio.

Selected organs were removed for isotopic analysis. Intestines were rinsed free of digesta with physiologic saline, and the entire carcass saved for isotopic analysis. Prior to isotopic analysis, carcasses were "cooked" in a microwave oven to permit separating the soft tissue from skeleton; each was then analyzed separately.

The data related to the exchangeable pool of Mg (Mg_e) in the three diet groups have been compared with the actual carcass Mg and its major components in Table 4.

Table 4- Comparison of Exchangeable Mg (Mg_e), Whole Carcass Mg and its Components in Animals Fed three Levels of Mg (EXP #2b)

Diet	Mg_e	Carcass Mg	Skeletal Mg	Soft Tissue Mg
	(mg/100 g body weight)			
D	16.6±0.6	26.1±0.2	11.6±0.1	14.5±0.2
C	27.2±1.7	30.4±0.4	14.5±0.2	15.9±0.3
H	44.4±8.9	33.0±0.7	16.3±0.8	16.7±0.4

The results given in Table 4 demonstrate that when dietary Mg is restricted on a chronic basis from the normal level (C to D), little decrease occurs in Mg content of soft tissues (9%) or carcass (14%), but somewhat more in skeletal Mg (20%). Considering the major decrease in dietary Mg (0.051% to 0.011%), the changes in tissue Mg content are modest. This is not surprising as the body must maintain its Mg, essential for myriads of biochemical reactions. However, this does point out an important limitation of tissue Mg measurements as measures of Mg status, even if such tissue were available for analysis (a major limitation in humans). In clear contrast, the predicted value of Mg_e (Table 4) based the concept of isotope dilution decreased by 39%, much higher than any of tissue Mg measurements. While the reasons for this large predicted change are not clear, they must be related to shrinking of the exchangeable Mg component of whole body Mg. In other words, in their efforts to conserve their Mg in the face of limited dietary supply, organs convert some of their Mg to non-exchangeable form. An alternative explanation would involve a change in affinity of different organs for newly absorbed (isotopic) Mg, effectively resulting in an apparent shrinking of the exchangeable pool. The latter explanation is

supported by the experimental evidence of differential isotope ratios upon institution of Mg-restriction (Fig. 2).

Regardless of the mechanisms involved, these data clearly demonstrate that a much larger change is predicted by the isotope dilution method than by tissue Mg analysis. Therefore, the former could be a much more sensitive method of assessing Mg status than the latter. This is especially important when it is realized that the magnitude of changes instituted in these animal model experiments with respect to dietary Mg are not likely to be encountered in humans. Rather humans are likely to encounter more subtle changes in body Mg, due to excessive sweating. Therefore, the need for a sensitive method that could predict small changes becomes apparent.

CONCLUSIONS

The work carried out to date under the auspices of this contract has led to the following important new methods. This is the first time that these methods have become available.

1- A method is now available to permit simultaneous measurement of total body water/extracellular fluid volume directly in humans. It involves administration of a cocktail containing both labeled water and NaBr, followed by sampling blood 3 hours after dosing.

2- A method has been developed which permits investigation of dynamics of Mg exchange in animal models. The method has, for the first time, been applied to measurement of differential organ affinity for Mg under the conditions of perturbed dietary Mg. The method appears to predict a larger change in exchangeable Mg than changes in tissue Mg analysis and may provide a more sensitive means of assessing Mg status.

3- A new method has been developed for accurate measurement of stable isotopes of K. This method is now being applied to the measurement of K_e (exchangeable K) in the animal model.

REFERENCES

1- Janghorbani M, and BTG Ting. Stable isotope tracer applications of ICP-MS. In: "Applications of Inductively Coupled Plasma Mass Spectrometry", Date, AR & Gray, AL, Blackie, London, 1989. pp. 115-140.

2- Janghorbani, M, TA Davis, and BTG Ting. Measurement of stable isotopes of bromine in biological fluids with inductively coupled

plasma mass spectrometry. Analyst, 113:405-411, 1988.

3- Schuette, SA, D Vereault, BTG Ting, and M Janghorbani. Accurate measurement of stable isotopes of magnesium in biological materials with inductively coupled plasma mass spectrometry. Analyst, 113:1837-1842, 1988.

4- Schuette, S, D Vereault, B Ting, and M. Janghorbani. In vivo studies of Mg exchange utilizing the stable isotope ^{25}Mg in the rat. Fifth Int. Mg Symposium, Kyoto, Japan, 8/8-12, 1988. In Press.

5- Schuette, S, J Hong, D Vereault, B Ting, and M. Janghorbani. Effect of Mg Nutriture on the dynamics of administered ^{25}Mg exchange in vivo in the rat. J Nutr, In Press.

6 - Ting, B., C.C. Lee, and M. Janghorbani. Measurement of Stable isotopes of potassium in biological matrices with inductively Coupled plasma mass spectrometry. Anal. Chem. In Press.